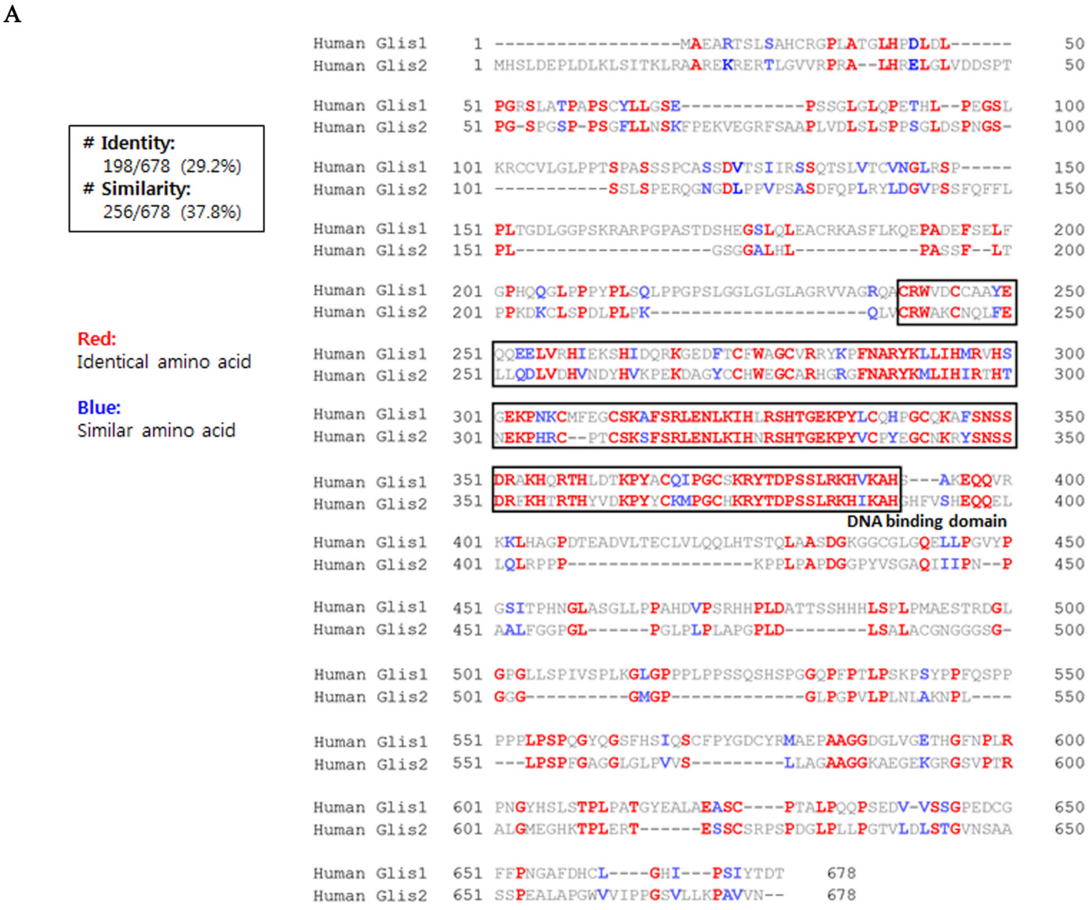
Glis family proteins are differentially implicated in the cellular reprogramming of human somatic cells

SUPPLEMENTARY MATERIALS



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Human Glis1     1 ------------------MAEARTSLSAHCRGPLATGLHPDLDL------     50
Human Glis2     1 MHSLDEPLDLKLSITKLRAAREKRERTLGVVRPRA--LHRELGLVDDSPT     50

Human Glis1    51 PGRSLATPAPSCYLLGSE------------PSSGLGLQPETHL--PEGSL    100
Human Glis2    51 PG-SPGSP-PSGFLLNSKFPEKVEGRFSAAPLVDLSLSPPSGLDSPNGS-    100

Human Glis1   101 KRCCVLGLPPTSPASSSPCASSDVTSIIRSSQTSLVTCVNGLRSP-----    150
Human Glis2   101 -----------SSLSPERQGNGDLPPVPSASDFQPLRYLDGVPSSFQFFL    150

Human Glis1   151 PLTGDLGGPSKRARPGPASTDSHEGSLQLEACRKASFLKQEPADEFSELF    200
Human Glis2   151 PL-------------------GSGGALHL------------PASSF--LT    200

Human Glis1   201 GPHQQGLPPPYPLSQLPPGPSLGGLGLGLAGRVVAGRQACRWVDCCAAYE    250
Human Glis2   201 PPKDKCLSPDLPLPK---------------------QLVCRWAKCNQLFE    250

Human Glis1   251 QQEELVRHIEKSHIDQRKGEDFTCFWAGCVRRYKPFNARYKLLIHMRVHS    300
Human Glis2   251 LLQDLVDHVNDYHVKPEKDAGYCCHWEGCARHGRGFNARYKMLIHIRTHT    300

Human Glis1   301 GEKPNKCMFEGCSKAFSRLENLKIHLRSHTGEKPYLCQHPGCQKAFSNSS    350
Human Glis2   301 NEKPHRC--PTCSKSFSRLENLKIHNRSHTGEKPYVCPYEGCNKRYSNSS    350

Human Glis1   351 DRAKHQRTHLDTKPYACQIPGCSKRYTDPSSLRKHVKAHS---AKEQQVR    400
Human Glis2   351 DRFKHTRTHYVDKPYYCKMPGCHKRYTDPSSLRKHIKAHGHFVSHEQQEL    400

Human Glis1   401 KKLHAGPDTEADVLTECLVLQQLHTSTQLAASDGKGGCGLGQELLPGVYP    450
Human Glis2   401 LQLRPPP------------------KPPLPAPDGGPYVSGAQIIIPN--P    450

Human Glis1   451 GSITPHNGLASGLLPPAHDVPSRHHPLDATTSSHHHLSPLPMAESTRDGL    500
Human Glis2   451 AALFGGPGL------PGLPLPLAPGPLD--------LSALACGNGGGSG-    500

Human Glis1   501 GPGLLSPIVSPLKGLGPPPLPPSSQSHSPGGQPFPTLPSKPSYPPFQSPP    550
Human Glis2   501 GGG----------GMGP-------------GLPGPVLPLNLAKNPL----    550

Human Glis1   551 PPPLPSPQGYQGSFHSIQSCFPYGDCYRMAEPAAGGDGLVGETHGFNPLR    600
Human Glis2   551 ---LPSPFGAGGLGLPVVS---------LLAGAAGGKAEGEKGRGSVPTR    600

Human Glis1   601 PNGYHSLSTPLPATGYEALAEASC----PTALPQQPSEDV-VSSGPEDCG    650
Human Glis2   601 ALGMEGHKTPLERT------ESSCSRPSPDGLPLLPGTVLDLSTGVNSAA    650

Human Glis1   651 FFPNGAFDHCL----GHI---PSIYTDT    678
Human Glis2   651 SSPEALAPGWVVIPPGSVLLKPAVVN--    678
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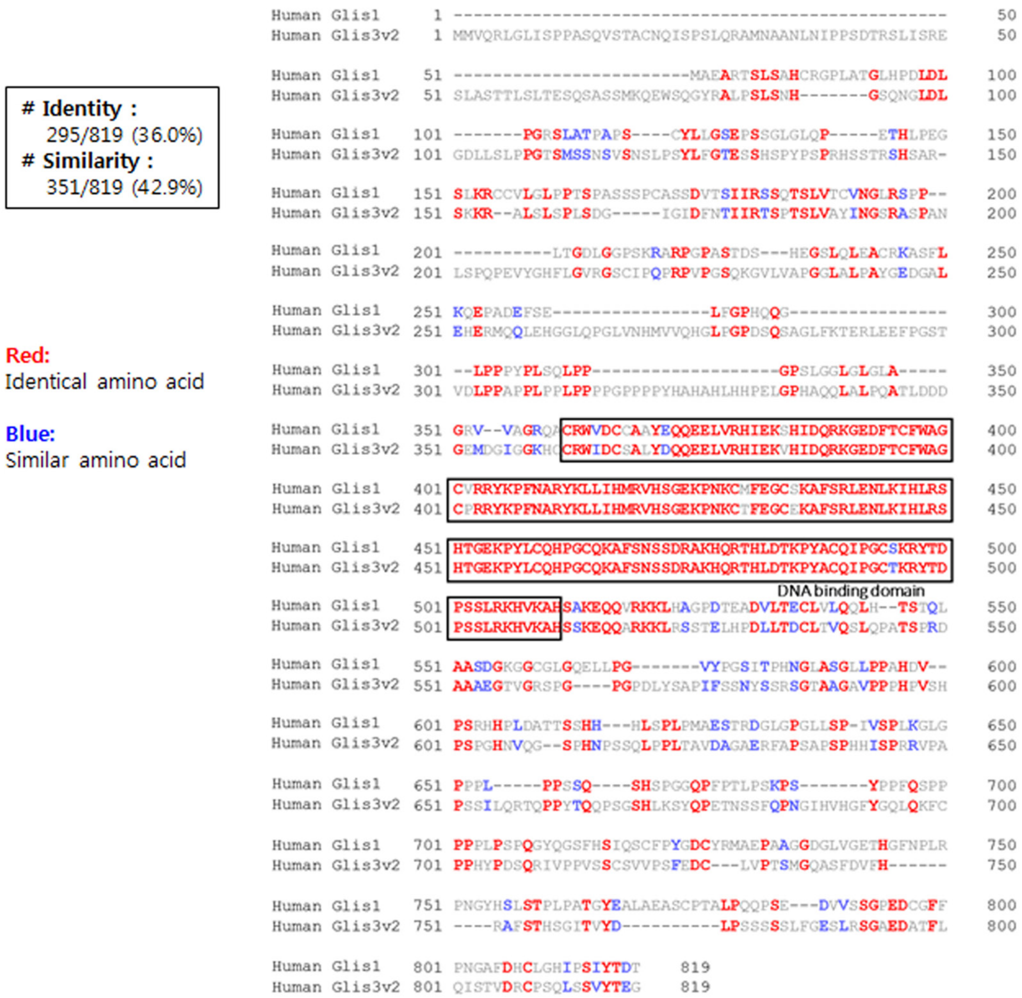
Supplementary Figure 1: The comparison of amino acid sequences between hGlis1 and hGlis2, and between hGlis1 and hGlisv2. (A) Amino acid sequence alignment between hGlis1 and hGlis2.

B



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Human Glis1     1 --------------------------------------------------    50
Human Glis3v2   1 MMVQRLGLISPPASQVSTACNQISPSLQRAMNAANLNIPPSDTRSLISRE    50

Human Glis1    51 ------------------------MAEARTSLSAHCRGPLATGLHPDLDL   100
Human Glis3v2  51 SLASTTLSLTESQSASSMKQEWSQGYRALPSLSNH-------GSQNGLDL   100

Human Glis1   101 -------PGRSLATPAPS----CYLLGSEPSSGLGLQP-----ETHLPEG   150
Human Glis3v2 101 GDLLSLPPGTSMSSNSVSNSLPSYLFGTESSHSPYPSPRHSSTRSHSAR-   150

Human Glis1   151 SLKRCCVLGLPPTSPASSSPCASSDVTSIIRSSQTSLVTCVNGLRSPP--   200
Human Glis3v2 151 SKKR--ALSLSPLSDG-----IGIDFNTIIRTSPTSLVAYINGSRASPAN   200

Human Glis1   201 ----------LTGDLGGPSKRARPGPASTDS---HEGSLQLEACRKASFL   250
Human Glis3v2 201 LSPQPEVYGHFLGVRGSCIPQPRPVPGSQKGVLVAPGGLALPAYGEDGAL   250

Human Glis1   251 KQEPADEFSE----------------LFGPHQQG----------------   300
Human Glis3v2 251 EHERMQQLEHGGLQPGLVNHMVVQHGLPGPDSQSAGLFKTERLEEFPGST   300

Human Glis1   301 --LPPPYPLSQLPP-------------------GPSLGGLGLGLA-----   350
Human Glis3v2 301 VDLPPAPPLPPLPPPPGPPPPYHAHAHLHHPELGPHAQQLALPQATLDDD   350

Human Glis1   351 GRV--VAGRQACRWVDCCAAYEQQEELVRHIEKSHIDQRKGEDFTCFWAG   400
Human Glis3v2 351 GEMDGIGGKHCCRWIDCSALYDQQEELVRHIEKVHIDQRKGEDFTCFWAG   400

Human Glis1   401 CVRRYKPFNARYKLLIHMRVHSGEKPNKCMFEGCSKAFSRLENLKIHLRS   450
Human Glis3v2 401 CPRRYKPFNARYKLLIHMRVHSGEKPNKCTFEGCEKAFSRLENLKIHLRS   450

Human Glis1   451 HTGEKPYLCQHPGCQKAFSNSSDRAKHQRTHLDTKPYACQIPGCSKRYTD   500
Human Glis3v2 451 HTGEKPYLCQHPGCQKAFSNSSDRAKHQRTHLDTKPYACQIPGCTKRYTD   500

Human Glis1   501 PSSLRKHVKAHSAKEQQVRKKLHAGPDTEADVLTECLVLQQLH--TSTQL   550
Human Glis3v2 501 PSSLRKHVKAHSSKEQQARKKLRSSTELHPDLLTDCLTVQSLQPATSPRD   550

Human Glis1   551 AASDGKGGCGLGQELLPG-------VYPGSITPHNGLASGLLPPAHDV--   600
Human Glis3v2 551 AAAEGTVGRSPG----PGPDLYSAPIFSSNYSSRSGTAAGAVPPPHPVSH   600

Human Glis1   601 PSRHHPLDATTSSHH---HLSPLPMAESTRDGLGPGLLSP-IVSPLKGLG   650
Human Glis3v2 601 PSPGHNVQG--SPHNPSSQLPPLTAVDAGAERFAPSAPSPHHISPRRVPA   650

Human Glis1   651 PPPL-----PPSSQ----SHSPGGQPFPTLPSKPS-------YPPFQSPP   700
Human Glis3v2 651 PSSILQRTQPPYTQQPSGSHLKSYQPETNSSFQPNGIHVHGFYGQLQKFC   700

Human Glis1   701 PPPLPSPQGYQGSFHSIQSCFPYGDCYRMAEPAAGGDGLVGETHGFNPLR   750
Human Glis3v2 701 PPHYPDSQRIVPPVSSCSVVPSFEDC---LVPTSMGQASFDVFH------   750

Human Glis1   751 PNGYHSLSTPLPATGYEALAEASCPTALPQQPSE---DVVSSGPEDCGFF   800
Human Glis3v2 751 ----RAFSTHSGITVYD----------LPSSSSSLFGESLRSGAEDATFL   800

Human Glis1   801 PNGAFDHCLGHIPSIYTDT     819
Human Glis3v2 801 QISTVDRCPSQLSSVYTEG     819
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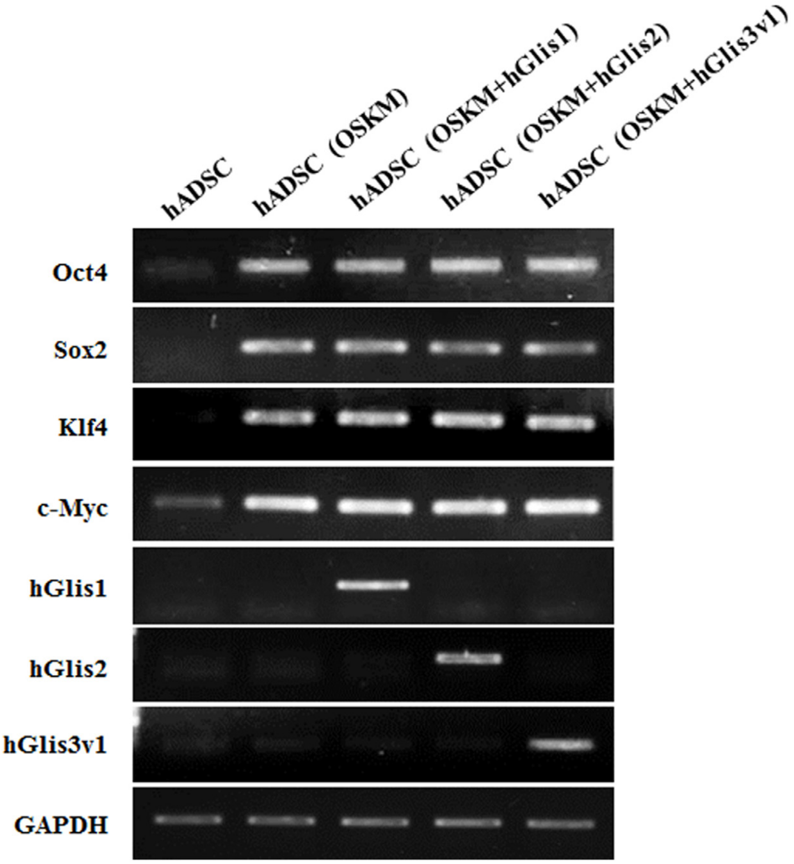
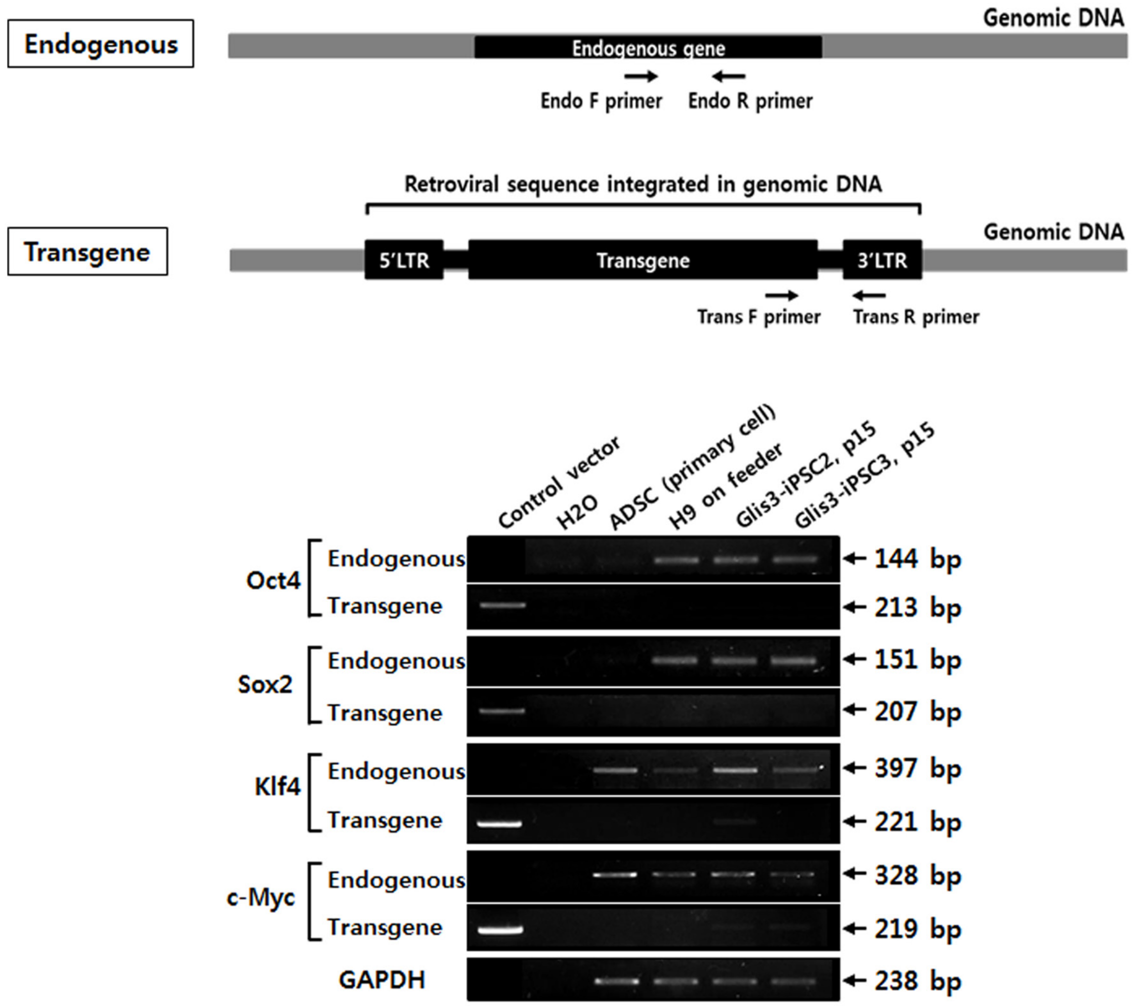
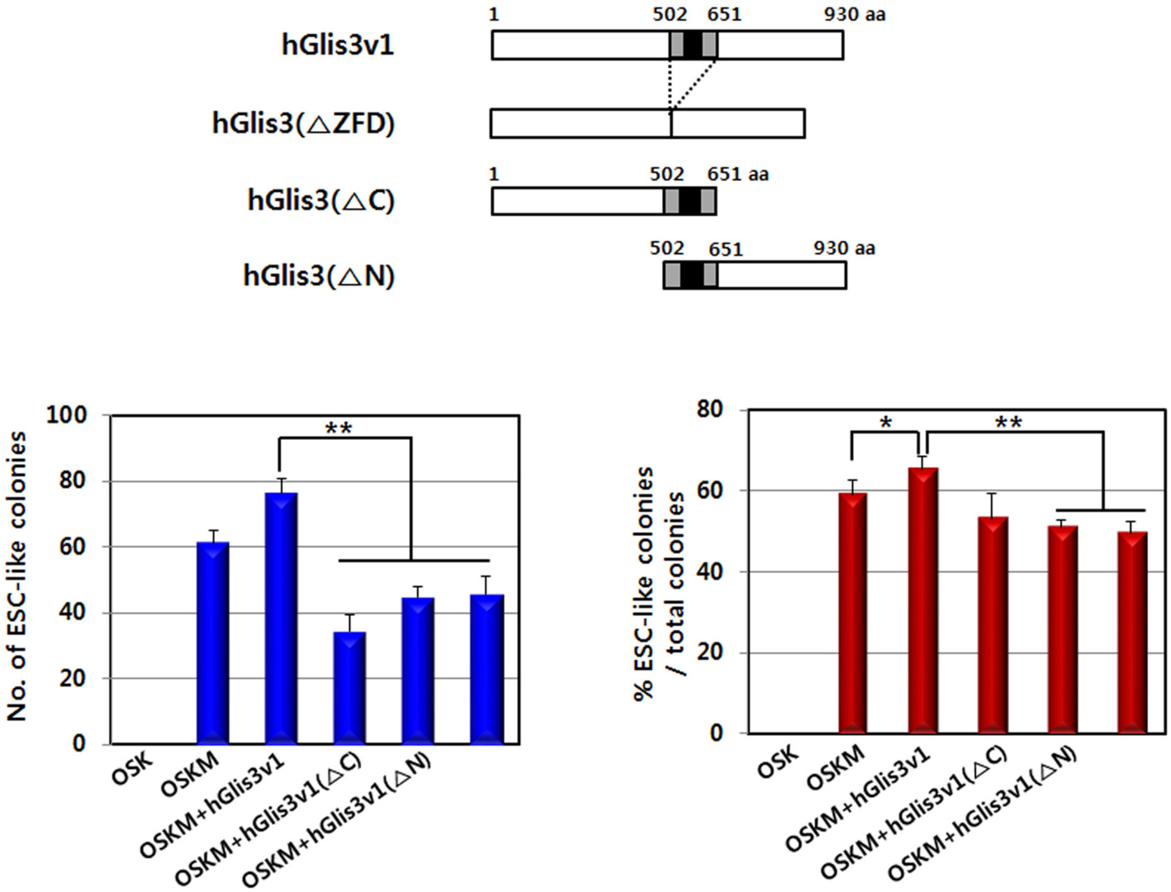
Supplementary Figure 1 (*Continued*): (B) Amino acid sequence alignment between hGlis1 and hGlis3v2.



Supplementary Figure 2: RT-PCR analysis of the expression of several reprogramming genes and Glis family genes. hADSCs were transduced with retroviral vectors carrying several reprogramming genes and Glis family genes, and the expression levels of the genes were examined 70 hours after transduction.



Supplementary Figure 3: RT-PCR shows the expression of exogenous and endogenous reprogramming genes in hADSCs, hESCs, and Glis3-iPSCs.



Supplementary Figure 4: Gene constructs for several truncated forms of hGlis3 proteins were generated and examined for their activities in promoting reprogramming efficiency. n=3, *$p<0.05$ **$p<0.01$.

Supplementary Table 1: List of the primer pairs used for the qRT-PCR and bisulfite sequencing analyses

Experiments	Gene	Forward primer (5’ - 3’)	Reverse primer (5’ - 3’)
qRT-PCR	Human Glis1	CACCAATAGTCAGCCCCCTG	GGCTCTGGAAGGGTGGGTAG
	Human Glis2	CATCCTGCGGACAGTTGTGG	CCAGCCATGAATGACCTAGC
	Human Glis3(v1+v2)	GAAGTGCCAGATGTGAGAAC	CAGACAGATGAGTGGATATC
	Human Glis3v1	CATCACATTCCTGCCATCCG	CGTTGTTCTGAGGAGCCATC
	Human β-actin	ATCTGGCACCACACCTTCTAC	GCGTACAGGGTAGCACAGC
	Human Oct4	AGAAGAGGATCACCCTGGGAT	AGAACCACACTCGGACCACAT
	Human Nanog	CCTGAAGACGTGTGAAGATGAG	GCTCATTAGGCTCCACCATAC
	Human Sox2	AGAAGAGGAGAGAGAAAGAAAGGGAGAGA	GAGAGAGGCAAACTGGAATCAGGATCAAA
	Human DNMT3B	GCTCACAGGGCCCGATACTT	GCAGTCCTGCAGCTCGAGTTTA
	Human Zic3	CAAAGTGTGCGACAAGTCCTACA	GGGAGGAATCTGACCCTTGAG
	Human Rex1	CAGATCCTAAACAGCTCGCAG	GCGTACGCAAATTAAAGTCCA
	Human NCAM	CACTATCTGGTCAGGTACCG	CCGCCTTGGATTTTCCTTGC
	Human Nestin	GAGAGCGAGCAGGAGGAGTT	GGAGTGGAGTCTGGAAGGGT
	Human Pax6	GCTTCACCATGGCAAATAACC	GGCAGCATGCAGGAGTATGA
	Human FoxF1	CACCAGAACAGCCACAAC	GCCATGGCGTTGAAAGAG
	Human Hand1	CACACTTGGATCGCACGT	GACAGTCCCTCCTTCTTG
	Human Gata2	CAGACGACAACCACCACCTTATG	TGGTCAGTGGCCTGTTAACATTG
	Human AFP	TTGGGCTGCTCGCTATG	TTTGTAACTGTTGCTGCCTTTG
	Human Gata6	TCCAGAAGCCAGGACTAGGA	TCCATGAACGCACATGAAAT
Bisulfite sequencing	Human Oct4	GGATGTTATTAAGATGAAGATAGTTGG	CCTAAACTCCCCTTCAAAATCTATT
	Human Nanog	AGAGATAGGAGGGTAAGTTTTTTTT	ACTCCCACACAAACTAACTTTTATTC

Supplementary Table 2: List of the antibodies used in this study

antibody (Host)	Company	Cat. No	Dilution
Oct4 (Mouse)	Santa Cruz	sc-5279	1:500
Sox2 (Rabbit)	Cell signaling	3579	1:500
SSEA4 (Mouse)	Millipore	MAB4304	1:500
Tra1-60 (Mouse)	Millipore	MAB4360	1:500
Tra1-81 (Mouse)	Millipore	MAB4381	1:500
Nestin (Mouse)	Millipore	MAB5326	1:200
Tuj1 (Mouse)	Covance	MMS-435P	1:200
SMA (Mouse)	AbFrontier	YF-PA23164	1:200
PECAM (Mouse)	Millipore	MAB1393	1:200
AFP (Mouse)	Abcam	Ab3980	1:200
FoxA2 (Mouse)	AbFrontier	YF-MA10439	1:200
Alexa Fluor 594 (Mouse)	Invitrogen	A-11005	1:500
Alexa Fluor 594 (Rabbit)	Invitrogen	A-11012	1:500
Alexa Fluor 488 (Mouse)	Invitrogen	A-11001	1:500
Alexa Fluor 488 (Rabbit)	Invitrogen	A-11008	1:500

Supplementary Table 3: List of the primer pairs used for RT-PCR analysis to distinguish the endogenous and transgene expression of the four Yamanaka reprogramming genes

Experiments	Gene	Forward primer (5’ - 3’)	Reverse primer (5’ - 3’)
Semiquantitative RT-PCR	Endogenous Oct4	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC
	Endogenous Sox2	GGGAAATGGGAGGGGTGCAAAAGAGG	TTGCGTGAGTGTGGATGGGATTGGTG
	Endogenous Klf4	ACGATCCTGGCCCCGGAAAAGGACC	TGATTGTAGTGCTTTCTGGCTGGGCTCC
	Endogenous c-Myc	GCGTCCTGGGAAGGGAGATCCGGAGC	TTGAGGGGCATCGTCGCGGGAGGCTG
	Transgene Oct4	CCCCAGGGCCCCATTTTGGTACC	ATTTTATCGTCGACCACTGTGCTG
	Transgene Sox2	GGCACCCCTGGCATGGCTCTTGGCTC	ATTTTATCGTCGACCACTGTGCTG
	Transgene Klf4	ACGATCCTGGCCCCGGAAAAGGACC	ATTTTATCGTCGACCACTGTGCTG
	Transgene c-Myc	CAACAACCCAAAATGCACCAGCCCCAG	ATTTTATCGTCGACCACTGTGCTG

Supplementary Table 4: DNA fingerprint analysis

Locus/sample	ADSC	Glis3-iPSC2(OSKM+hGlis3)
D8S1179	**10, 13**	**10, 13**
D21S11	**31, 33.2**	**31, 33.2**
D7S820	**9, 11**	**9, 11**
CSF1PO	**11, 11**	**11, 11**
D3S1358	**15, 17**	**15, 17**
TH01	**7, 9**	**7, 9**
D13S317	**8, 11**	**8, 11**
D16S539	**9, 10**	**9, 10**
D2S1338	**20, 20**	**20, 20**
D19S433	**14, 14**	**14, 14**
vWA	**18, 18**	**18, 18**
TPOX	**8, 11**	**8, 11**
D18S51	**13, 16**	**13, 16**
D5S818	**11, 11**	**11, 11**
FGA	**22, 25**	**22, 25**

Supplementary Table 5: List of the hESC-enriched genes

See Supplementary File 1

Supplementary Table 6: List of the hADSC-enriched genes

See Supplementary File 1